Inherited Thrombophilia and Pregnancy Loss

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Introduction

A successful pregnancy is dependent on the development of adequate placental circulation. Abnormalities of placental vasculature may result in a number of gestational pathologies, including first and second trimester miscarriages, intrauterine growth retardation (IUGR), intrauterine fetal death (IUFD), placental abruption, and preeclampsia.1

Approximately 5% of women experience two or more consecutive abortions. Habitual abortions, defined as three or more spontaneous recurrent pregnancy losses, may affect as many as 1% to 2% of women of reproductive age. The discovery of an association between recurrent pregnancy loss, and antiphospholipid antibodies, specifically lupus anticoagulant, and anticardiolipin antibodies increased interest in a possible acquired thrombotic autoimmune cause.

The inherited thrombophilias are a group of genetic disorders of blood coagulation resulting in an increased risk of thrombosis. Today, a full understanding of the inherited thrombophilias is becoming increasingly important in the management of high-risk gestations. Several reports over the last three years have suggested that not only are these disorders associated with an increased risk of thromboembolic disease during pregnancy and puerperium, but they are also associated with an increased incidence of vascular pathologies, resulting in poor gestational outcome.2

This review will cover recent data concerning thrombophilia and vascular placental pathology, potential pathophysiologic mechanisms for this association, and available therapeutic modalities for prevention of placental vascular thrombosis in order to maximize successful gestational outcome.

Recurrent Pregnancy Loss

Recurrent pregnancy loss (RPL) is a common health problem affecting 1% to 2% of women at the reproductive age.3,4 Several etiologies have been implicated to play a role in RPL. These include chromosomal translocations and inversions, anatomic alterations of the uterus, endocrinologic abnormalities, and autoimmune disorders.5,6 However, until recently, the majority of RPL remained unexplained.

RPL is a well-established finding in certain acquired thrombophilic disorders, such as antiphospholipid syndrome7 and essential thrombocythemia.8 Several earlier reports demonstrated an increased thrombotic risk during gestation and puerperium in women with an inherited thrombophilic disorder, such as deficiencies in antithrombin III, protein C, or protein S.9,10 More recently, a case control study in 60 women with these inherited thrombophilias documented an increased risk for RPL as well.11 Forty-two out of 188 pregnancies (22%) in women with thrombophilia resulted in pregnancy loss compared to 23/202 (11%) in controls (OR=2.0, 95% CI 1.2-3.3).11 In addition, a high incidence of gestational abnormalities was reported in 15 women with dysfibrinogenemia associated with thrombosis. Of 64 pregnancies, 39% ended by miscarriage and 9% by intrauterine fetal death.12

Certain women with RPL exhibit hypofibrinolysis related to abnormal plasma levels of fibrinolysis activators and inhibitors. It appears that these patients may have functional abnormalities of the vascular endothelium characterized by high plasma levels of von Willebrand Factor (vWF), tissue plasminogen activator (t-PA), and plasminogen activator inhibitor-1, which may be associated with increased thrombin formation.13,14 These abnormalities may lead to poor placental implantation or to a very early insufficiency of the fetal-maternal circulation. It has been suggested that antithrombotic therapy to reduce thrombin formation could allow the reestablishment of a favorable hemostatic balance and would encourage not only early placentaion, but potentially, successful full-term gestation.13,14

Factor V Leiden mutation, factor II G20210A mutation, and hyperhomocysteinemia account for the majority of venous thromboembolic events, particularly during gestation or in association with oral contraceptive use. Factor V Leiden mutation can be found in over 50% of women with gestational thrombosis.15 Factor II G20210A mutation is associated with a 20% to 50% increase in prothrombin plasma levels and a three-fold increased risk for venous thrombosis.16

A founder effect has recently been suggested to account for the relatively high prevalence of factor V Leiden and factor II G20210A mutations in Caucasian populations.17,18 The high prevalence of these mutations in Caucasians and the worldwide high prevalence of hyperhomocysteinemia and homozygosity for the methylenetetrahydrofolate reductase (MTHFR) C677T mutation19,20 set the stage for studies of these common thrombophilic states in women with RPL.

Transient activated protein C (APC) resistance can be documented during normal gestations in women with normal factor V genotype. The APC-sensitivity ratio shows a progressive fall during normal pregnancy in correlation with changes in factor VIII, factor V, and protein S levels.21 APC-sensitivity ratios may decrease further during gestation in women with factor V Leiden mutation.

Likewise, APC-sensitivity ratios were reported to be decreased in patients with RPL.22-25 While APC-resistance is more common in women with second trimester losses,22 it can...
also be found in women with first trimester RPL. Indeed, APC-resistance was documented in 20/41 (49%) of women experiencing second trimester RPL compared to only 10/37 (27%) of women experiencing first trimester abortions (p<0.05). Several case studies have suggested a potential association between factor V Leiden and RPL, while others have not found such a correlation. These discrepancies may be explained, in part, by differences in selection criteria, including the ethnic origins of the study populations. For example, the European Prospective Cohort on Thrombophilia (EPCOT) study analyzed the risk for fetal loss in a cohort of 571 women with known inherited thrombophilias of various types. In this study, the researchers found that the odds ratios were 3.6 (95% CI 1.4-9.4) for stillbirths and 1.3 (95% CI 0.94-1.71) for miscarriages. For women with factor V Leiden mutation, in particular, the odds ratios were 2.0 (0.5-1.77) for stillbirth and 0.9 (0.5-1.5) for miscarriages.

Three recent case control studies, summarized in Table 1, have evaluated the prevalence of factor V Leiden mutation in women with RPL. Despite differences in Caucasian subpopulation and selection criteria for RPL, all three studies documented a significantly increased prevalence of factor V Leiden mutation in women with RPL. Ridker et al have studied women with RPL who had not undergone an extensive etiological work-up, except for ruling-out chromosomal abnormalities. They found a 2.3-fold increased prevalence of factor V Leiden mutation in women with RPL.

Other potential causes for RPL, including chromosomal abnormalities, infection, anatomic alterations, endocrinologic abnormalities, and autoimmune disorders, should be eliminated. In women without these apparent causes for RPL, however, studies by Grandone et al and Brenner et al have suggested that evaluation of factor V Leiden mutation is highly warranted, as a significant percentage of women with RPL are found to be carriers of the mutation. In populations where factor V Leiden homozygosity is highly prevalent, significant association with RPL can also be demonstrated.

A potential interpretation of these four studies is that factor V Leiden, which is a mild risk factor for thrombosis, is also a mild risk factor for RPL. In other words, although it may play a role in RPL, the majority of women who are carriers of the mutation will not experience RPL. The prevalence of the mutation in the general population will determine, in part, its prevalence in women with RPL.

The role of factor II G20210A mutation in RPL was recently evaluated in three studies. The study by Pickering et al demonstrated no difference in factor II G20210A prevalence between women with RPL and controls (4.4% versus 4.5%). Likewise, Deitcher et al studied 50 patients with first trimester RPL and found one individual (2%) with the factor II mutation. The third study demonstrated an odds ratio of 2.2 (95% CI 0.6-8.0, p=0.23) for factor II G20210A in the RPL group compared to controls. However, these small-scale studies cannot rule out the possibility that factor II G20210A mutation is a mild risk factor for RPL.

Plasma homocysteine levels decrease during normal pregnancy compared to levels in non-pregnant women. Several recent studies have shown that homozygosity for the MTHFR C677T mutation is not predictive for RPL, while other studies reported a potential association between RPL, hyperhomocysteinemia, and homozygosity for MTHFR C677T. Moreover, plasma homocysteine levels can increase in pregnant women with folic acid or vitamin B12 deficiency, particularly in the presence of homozygosity for MTHFR C677T, and this may result in RPL.

It is well established that combinations of inherited or acquired thrombophilic states increase the risk for thrombosis. Likewise, combinations of thrombophilic states may further increase the risk for RPL. For example, coexistence of factor V Leiden and homozygous hyperhomocysteinemia or a combination of factor V Leiden with familial antithrombopilid syndrome were reported to result in thrombosis and recurrent fetal loss. It is, therefore, not surprising that the EPCOT study documented the highest odds ratio for stillbirth (OR=14.3, 95% CI 2.4-86) in patients with combined thrombophilic defects. In our recent study involving 76 women with RPL, six (8%) had combined thrombophilia compared to 1/106 (0.9%) of controls (p<0.02).

In view of the high prevalence of the three common thrombophilic mutations, factor V Leiden, factor II G20210A, and MTHFR C677T, in the general population, we have evaluated their prevalence in women with RPL. At least one was found in 49% of women with RPL of unknown cause compared to 23% in controls (OR=3.2, 95% CI 1.7-6.1, p=0.0002). Without therapeutic intervention, less than 20% of gestations in women with thrombophilia and RPL result in live birth. This is similar to rates reported in women with the antiphospholipid syndrome who experience RPL. Although the majority of pregnancy losses are in the first trimester, women with thrombophilia have an increased percentage of losses at later stages of gestation. For example, second trimester losses or IUFD accounted for 57/158 (36%) of pregnancy loss in 37 women with thrombophilia compared to only 23/135 (17%) in women with RPL without thrombophilia (p=0.0004).

Other Placental Vascular Complications

A variety of other placental vascular complications may be associated with thrombophilia, as shown in Table 2.
Table 2. Placental Vascular Complications Associated with Thrombophilia

<table>
<thead>
<tr>
<th>Inherited Thrombophilia</th>
<th>Miscarriages</th>
<th>IUFD</th>
<th>Preeclampsia</th>
<th>HELLP</th>
<th>Placental Abruption</th>
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<tbody>
<tr>
<td>Antithrombin III Deficiency</td>
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<td>Protein C Deficiency</td>
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<td>MTHFR C677T</td>
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<tr>
<td>Combined Defects</td>
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</tbody>
</table>

IUFD—Intra-uterine fetal death
HELLP—Hemolysis, elevated liver enzymes, low platelet counts

Degree of Association
+ Possible association
++ Established association

Preeclampsia, characterized by gestational hypertension, edema, and proteinuria, has long been associated with an abnormal placental vasculature. Activation of blood coagulation and endothelial cell stimulation are fundamental findings in preeclampsia. Activation of the coagulation and fibrinolytic systems is more marked in the uteroplacental circulation than in the systemic circulation, and an abnormal pattern of hemostasis has been reported to operate in the uteroplacental circulation in women with preeclampsia.

Several reports have suggested an association between APC-resistance, factor V Leiden mutation, and early onset of severe preeclampsia. In one study, 14 of 158 (9%) women with severe preeclampsia were found to be heterozygous for the factor V Leiden mutation compared with 17 of 403 (4%) normotensive pregnant women serving as controls (p=0.03). Likewise, in another study, factor V Leiden mutation was documented in 19% of women with preeclampsia compared to 7% in the control group.

One severe presentation of preeclampsia, termed HELLP syndrome, is manifested by hemolysis, elevated liver enzyme levels, and a low platelet count. A potential association between factor V Leiden mutation and HELLP syndrome has been reported in two women. Therapy with low molecular weight heparin throughout three successive pregnancies resulted in normal delivery. Another study has documented APC-resistance in 7/21 women with HELLP syndrome. In 85 women with a history of severe early-onset preeclampsia, Dekker et al have found a variety of thrombophilic defects, including protein S-deficiency (25%), anticardiolipin antibodies (29%), APC-resistance (16%), and hyperhomocysteinemia (18%). Thirteen patients had combinations of thrombophilic defects, emphasizing the role of combined thrombophilia in the observed placental vascular pathology. Interestingly, 53% of women with severe early-onset preeclampsia in Dekker’s report had HELLP syndrome. Recently, Grandone et al have shown a predisposition for preeclampsia in women with either factor V Leiden or MTHFR C677T mutations.

An association between vascular placental complications and hyperhomocysteinemia has increasingly been reported. Hyperhomocysteinemia was documented in 26% of women with placental abruption, 11% of the cases with IUFD, and 38% of women delivering babies with birth weights below the fifth percentile compared to an estimated 2% to 3% in the general control population. Likewise, hyperhomocysteinemia was documented in 26/84 (31%) women with previous placental infarcts or abruption compared to 4/46 (9%) in controls. In the Hordaland Homocysteine Study, plasma homocysteine levels were evaluated in 5,883 women with 14,492 gestations. The study, which is the largest performed to date, reported an increased risk for preeclampsia (OR=1.33), stillbirth (OR=2.11), early labor (OR=1.41), and placental abruption (OR=3.03).

APC-resistance and factor V Leiden have recently been associated with placental abruption. Seventeen out of 27 (63%) women with placental abruption had APC-resistance compared to 5/29 (17%) controls (OR=8.2, 95% CI 3.6-12.7). Factor V Leiden was documented in 8/27 (30%) of patients compared to 1/29 (3%) of controls.
The association of the genetic thrombophilias with gestational vascular complications has been evaluated in 110 women with preeclampsia, intrauterine growth retardation, placental abruption, or stillbirth, as compared to 110 women with normal gestations. One of the three common thrombophilic mutations, factor V Leiden, factor II G20210A, or MTHFR C677T, was found in 57/110 (52%) of women with gestational vascular complications compared to only 19/110 (17%) of controls (OR=5.2; 95% CI 2.8-9.6). Additional patients had other thrombophilias accounting for a total of 71/110 (64%) compared to only 20/110 (18%) in controls (p<0.001). Patient and control groups differ in parity with 92/110 of patients being in their first pregnancy compared to only 62/110 of controls (p<0.001).

The three inherited thrombophilias were more common in women with severe preeclampsia (OR=5.4, 95% CI 2.3-12.4), placental abruption (OR=7.2, 95% CI 2.3-20), intruterine growth retardation (OR=4.8, 95% CI 2.2-10.3), and stillbirth (OR=3.4, 95% CI 1.0-11.9). These gestational vascular complications resulted in earlier delivery, 32 weeks versus 39 weeks in controls, and decreased birth weight of 1,375 g versus 3,400 g in controls.

Because up to 65% of vascular gestational abnormalities can be accounted for by genetic thrombophilias, the implication is to screen for these mutations in all women with vascular gestational abnormalities. Furthermore, this high prevalence of genetic thrombophilias, which is similar to the findings in women with pregnancy-related venous thromboembolism, suggests that antithrombotic drugs may also have potential therapeutic benefit in women with gestational vascular complications.

**Therapeutic Regimens**

Recently, two prospective randomized studies have shown that heparin plus low-dose aspirin results in significantly better gestational outcome than low-dose aspirin alone in women with antiphospholipid syndrome who had experienced recurrent pregnancy loss. In a study conducted by Kutteh et al viable infants were delivered in only 11/25 (44%) of women receiving aspirin compared to 20/25 (80%) in women receiving aspirin and subcutaneous heparin (p<0.05). In a study by Rai et al, the rate of live birth in patients treated with low-dose aspirin and heparin was 71% (32/45 pregnancies) compared to only 42% (19/45 pregnancies) in women treated with aspirin alone (p<0.01). It should be emphasized that despite the progress obtained by current therapeutic regimens, complications still occur in a significant number of pregnant women with antiphospholipid syndrome. In the study conducted by Rai et al, one-fourth of successful pregnancies were delivered prematurely, suggesting that the optimal therapeutic regimen has not yet been established.

Emerging data on therapy of women with inherited thrombophilia and pregnancy loss were collected primarily in uncontrolled studies involving only small series of patients. The role of low molecular weight heparin (LMWH) in this setting deserves study in prospective clinical trials. The potential advantages of LMWH over unfractionated heparin are higher antithrombotic ratio, meaning less bleeding for better antithrombotic effect, longer half-life with a potential need for only one injection per day, smaller injected volume, and less heparin-induced thrombocytopenia. A recent collaborative study has demonstrated the safety of using LMWH during 486 gestations. Successful outcome was reported in 83/93 (89%) gestations in women with recurrent pregnancy loss and in all 28 gestations in women with preeclampsia during a previous pregnancy. Administration of Enoxaparin 20mg/day to women with primary early RPL and impaired fibrinolytic capacity resulted in normalization of impaired fibrinolysis, conception in 16/20 (80%), and successful live birth in 13/16 (81%).

During the past four years, we have treated 63 pregnancies in 42 women with thrombophilia who presented with thromboembolism and/or RPL with LMWH (Enoxaparin, Rhone Poulenc, France) throughout gestation (unpublished data). The dosage of LMWH used was 40 mg/day, except for patients with combined thrombophilia or abnormal Doppler velocimetry suggesting decreased placental perfusion, where the dosage was increased to 40 mg bid aiming toward an anti-factor Xa level of 0.3-0.4 Xa units. Plasma anti-factor Xa levels may decrease in late pregnancy, implying a potential need for LMWH dosage increase at that stage. In the case of previous thrombosis, LMWH therapy was continued for six weeks after delivery. Forty-five of the 63 pregnancies (71%) resulted in live birth. In women with inherited thrombophilia and RPL, the percentage of live births increased from 20% without therapy to 75% following LMWH treatment.

A beneficial effect of aspirin in secondary prevention of preeclampsia has been suggested. However, these observations have not been confirmed by more recent studies. Recent preliminary reports suggest that LMWH, with or without aspirin, has a beneficial role in women with thrombophilia and vascular gestational abnormalities, including preeclampsia, and fetal growth retardation.

The role of aspirin in the setting of thrombophilia and vascular gestational abnormalities remains to be confirmed. In patients with antiphospholipid syndrome, or in those with combined thrombophilia, aspirin is given along with LMWH. However, whether aspirin has an added benefit to heparin or LMWH alone has not been evaluated. Prospective, randomized, dose-finding studies are warranted to assess the potential advantage of LMWH in women with thrombophilia and vascular gestational abnormalities.

The relative effectiveness of some therapeutic modalities for prevention of pregnancy loss in thrombophilic patients is summarized in Table 3.

**Future Perspectives**

Future research is this field will most likely deal with four specific aspects. First, the potential associations between various genetic thrombophilias and gestational vascular pathologies will require verification.

Second, currently 30% to 50% of vascular gestational pathologies cannot be accounted for by any of the known
Table 3. Therapeutic Modalities for Prevention of Pregnancy Loss in Thrombophilic Patients

<table>
<thead>
<tr>
<th>Inherited Thrombophilia</th>
<th>Steroids</th>
<th>Aspirin</th>
<th>Heparin</th>
<th>LMWH</th>
<th>Factor Concentrates</th>
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<td>Factor V Leiden</td>
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<td>Combined defects</td>
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</table>

**Therapeutic Benefit**

+       Equivocal  
++      Substantial  
+++     High

thrombophilias. Whether novel genetic mutations or acquired thrombophilia will be found to play a role remains to be determined. Of interest, recent observations suggest that the presence of anti-endothelial cell antibodies63 and complement-fixing antibodies to trophoblasts64 may be associated with RPL. These studies, as well as those suggesting presence of antibodies directed toward specific phospholipids, or inhibiting protective molecules such as annexin V65 imply a broader spectrum for RPL related to autoantibodies. Whether the acquired non-genetic APC-resistance observed in women with RPL23-24,66 is related to autoantibodies remains to be determined.

Third, the pathogenetic mechanisms responsible for placental vascular pathologies in women with thrombophilia have not been elucidated. Furthermore, it is yet unknown why only some women with thrombophilia express vascular gestational pathologies, while others do not. It is possible that this may relate to local factors affecting coagulation, fibrinolysis, and vascular tone at the level of placental vasculature.

Finally, the role of antithrombotic therapeutic modalities deserves prospective clinical trials to improve outcome for a large population of women who currently poor gestational outcome.

References


